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Dr. James Ewing, *President.*

A CASE OF BOTHRIOCEPHALUS, WITH REMARKS ON THE OCCURRENCE OF BOTHRIOCEPHALUS IN AMERICA.

W. N. BERKELEY, M.D.

Dr. Berkeley presented specimens from a case of Bothriocephalus. The patient was a native of Russia, forty-six years of age, a female, married, and had several children. She came to this country a few years ago. She had been passing segments of the worm for eight years, but had never had medical advice. She was in good health, had no anemia, and complained only of vague digestive complaints. Treatment was begun and two

days later she brought the worm with the head complete to the dispensary. Dr. Berkeley said that he had been able to find no references in the literature to any cases endemic in the United States. Ritchie in his recent article on Parasites *(Encyclopedia Medica)* said that the worm was "seldom seen in England", but made no mention of cases in this country. Dock had observed three cases in this country, one in Colorado and two in Texas, both of which he was satisfied were imported. Osler also noted that there were no endemic cases. In the last edition of *Braun's Thierische Parasiten* the name had been restored to its older form, Dibothriocephalus. Braun could mention only a single case from the United States, not stating whether this was believed to be endemic or imported. Dr. Berkeley had observed three other cases in the last four years, all in the same class of Russian immigrants. In these cases there were no signs of anemia and the patients complained only of indefinite digestive disorders. The parents of one patient, a small girl, claimed that she had never passed segments of the worm until three months before consulting the doctor, and years after her arrival in this country. On examination it was decided, however, that this was probably not correct, and the case was classified as an imported one.

Discussion.

Dr. Theodore C. Janeway said that he had seen a case of Bothriocephalus during the week in a woman from Finland. She was in good health; hemoglobin was eighty per cent. Her symptoms were similar to those of Dr. Berkeley's case. She had noticed passing the first segments of the worm eight years before, but said she had not seen them again until recently. The worm obtained was twenty-four feet in length.

Dr. James Ewing said that some years ago he had presented to the Society three large specimens all taken from the same subject, a Swede, who claimed not to have eaten raw fish for seventeen years. He had been entirely well and had had no signs of anemia.

DEMONSTRATION OF THE HEMOSIDERIN REACTION IN CORNEAL VACCINE BODIES.

JAMES EWING, M.D.

Dr. Ewing said that about six months before he had had occasion to bring together some evidence regarding the nature of vaccine bodies. This evidence pointed to the view that red blood cells sometimes assumed the position and exhibited the staining qualities of vaccine bodies. He had not claimed that all vaccine bodies were of this nature, nor did he wish to go so far yet. He merely wished to present some evidence which he had obtained in favor of this theory. The hemosiderin reaction he had not been able to get in every instance; in fact, in the majority of cases the stain had been a failure, and in some sections he had succeeded in getting the blue stain in only a portion of the vaccine bodies. The specimens were hardened in bichloride saturated in .6% salt solution, cut in paraffin and allowed to remain twenty-four hours in potassium ferrocyanide, and six hours in hydrochloric acid. The blue color was seen only in the center of the vaccine body and by staining with eosin or safranin the outer portion of the body took on a red stain. In many sections of human tissues the

vaccine bodies gave only a faint diffuse reaction. The meaning of the blue stain thus obtained in the center of the vaccine bodies he was unprepared to discuss. He thought some would regard it as evidence that these bodies contained free iron and were derivatives of red cells. Of course, the fact that the stain was not obtained in every instance was a suspicious circumstance.

LIMITS OF A SPECIFIC REACTION IN THE SERUM TEST FOR BLOOD.

JAMES EWING, M.D., AND ISRAEL STRAUSS, M.D.

Dr. Ewing presented tables showing the results of experiments, conducted with Dr. Israel Strauss, relating to the limits of a specific reaction in the serum test for blood; that is, the test by which it was believed to be possible to distinguish between all varieties of blood. He said it was already known that reactions among so-called specific anti-sera were only quantitative and not strictly specific. The specific anti-body developed in the serum of the rabbit treated with human blood would produce a reaction in a variety of other bloods as well as in human blood, Only at a certain point of dilution did the heterologous bloods drop out, leaving the reaction only in the human blood. It was therefore very necessary to determine the solutions of blood with which one worked in order to estimate results correctly. One series of experiments were made on the effects of the serum of a rabbit, which had received multiple injections of beef blood, on various other bloods. The bloods tested were human, goat, cat, chicken, sheep, horse and beef. Various dilutions were used, from 1–10 to 1–1500. When the

serum was added to all these different bloods in a dilution of one part of serum to five parts of blood, practically the same results were obtained in all. At the end of thirty minutes there was a flocculent precipitate in human blood; the reactions in beef and goat blood were more distinct than in the other, and it was not possible to distinguish the beef blood. In a dilution of serum of one to ten, human blood gave little reaction; chicken, horse and rabbit bloods gave no reaction at all. The beef and goat were about the same. At one to fifteen, human blood dropped out entirely. The sheep still reacted. In the lower dilutions it had been about the same as the goat. Comparison of the beef and goat at one to fifteen showed rather in favor of the goat. At one to twenty, sheep blood still gave a distinct reaction, being turbid at the end of three hours. The beef gave a flocculent precipitate. At one to thirty, the sheep blood dropped out; beef and goat blood each gave a prominent turbidity, about equally strong. At one to fifty, the beef blood reacted as strongly as ever, but the goat blood had dropped out almost entirely. This showed that the reaction was specific only in high dilutions. In order to get a specific reaction with the beef-rabbit serum, it was necessary to dilute the serum up to one to fifty. The dilution of the serum added seemed to be more important than the dilution of the blood to be tested. Experiments were then made with humanized rabbit serum. Two sera were tested, one prepared by Dr. Strauss, and one by Dr. Paine. With Dr. Strauss' serum tests were first made at a dilution of one to five. There was no reaction with dog, cat or horse serum. There was a slight reaction in sheep, beef and goat bloods. The reaction in human blood, however, was not sufficiently distinct.

At one to ten goat blood dropped out. The sheep blood still reacted, but in diminished intensity. Human blood was the same. The beef reaction was slightly diminished. At one to twenty, there was nothing left but the very prominent reaction in human blood. This seemed to be encouraging for the specificity of the test for human blood until the other serum was tested, when different results were found. With this serum at one to five, the sheep, beef and human bloods showed good reactions. There was nothing with dog, cat or horse. This serum was evidently stronger than the first. At one to ten, human, sheep, beef, and goat blood still showed about the same reaction. At one to twenty, the reaction in the human blood was much the most distinct, but the reaction in sheep and beef blood was still quite as distinct as some obtained in human blood with the other serum. These results showed that the dilutions of this particular serum must be made above one to twenty before one could eliminate all but human blood. Some of Dr. Ewing's conclusions were; that it was unsafe to give credence to anything except a flocculent precipitate, that the time in which this flocculent precipitate occured was of the greatest importance. Before two hours it never occurred in his experiments in any blood except that against which the serum was prepared, if the test serum had been diluted at least one to twenty. It would seem that reliable results could not be assured unless the test serum was diluted at least one to fifty.

Discussion.

Dr. W. H. Park asked whether Dr. Ewing knew what the rabbit serum would have done before the injections of human blood. He considered that it was very im-

portant to know the agglutinating properties of the blood before treatment, so as to judge how much of the agglutination was due to the normal blood, and how much to the influence of the human or other blood injections.

DR. EWING said that he realized the importance of Dr. Park's statements. This work had only been done as a beginning. At the time he had himself thought it very important to know what the animal's blood would do before the experiments were made. In the first case, weak dilutions were practically inert in all the bloods tested.

DR. F. C. WOOD asked Dr. Ewing whether the strength of the dilutions of the blood to be tested were determined with accuracy or whether they were merely roughly determined.

DR. EWING said that the dilutions of blood had been determined with great accuracy, as was shown by the tables. A more important point, however, was the strength of the serum.

DR. OTTO H. SCHULTZE asked whether in tests made with humanized rabbit serum there had not been some differences which the tables shown had not brought out. Dr. Ewing had said that in the various dilutions all the reactions were practically the same. Had there not been some difference in the amount of the precipitate dependent upon the strength of the dilution used?

DR. EWING said that the stronger the dilutions of the blood the larger in most instances were the amounts of precipitates obtained.

DR. S. J. MELTZER asked whether Dr. Ewing had followed out these tests with regard to the character of the food of the animals he experimented with. It would

appear that his animals could be classified into two groups; carnivorous and herbivorous animals. It appeared to him quite plausible to assume that the proteids of the bloods of animals of the same group had among themselves a greater similarity than that between the animals of different groups. It would therefore be not unreasonable to expect to find that the precipitin reactions were less specific with the blood of animals which were fed in the same manner than with the blood of animals which were fed differently.

DR. EWING said that he had not yet got to this point. Nuttall he knew, had attempted to classify animals by the serum reactions. He himself did not believe that the subject was in such a position that it was possible to draw reliable conclusions of this character. Investigators were in no position to eliminate just now all the disturbing results of the peculiarities of the particular animals with which they worked. He had, with the other sera, tested the serum of a chicken immunized against rabbit blood, which acted so peculiarly that he had thought it best not to tabulate the results.

A STUDY OF A BACILLUS RESEMBLING THE BACILLUS OF SHIGA, FROM A CASE OF FATAL DIARRHŒA IN A CHILD; WITH REMARKS ON THE RECOGNITION OF DYSENTERY, TYPHOID, AND ALLIED BACILLI.

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AND

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Several reasons have led us to report the results of our study of the organism isolated from this case of fatal diarrhœa. Chief among these is the vital importance of establishing beyond doubt the etiology of the various forms of diarrhœas and dysenteries. Upon such establishment of etiology must ultimately depend the rationale of hygienic, diagnostic, and therapeutic measures —especially those of serum therapy, and diagnosis by serum reactions—upon which the recognition and cure of the disease or protection from the infective agent must be based. Moreover, there is the greatest need, in the present state of our knowledge of the etiology of dysentery, of the most careful study, description, and especially comparison of the bacterial forms supposed, in different cases and various localities, to be of etiological significance in the same or various forms of this disease.

As will be brought out more fully in detailing the peculiarities of the organism we are about to describe, the organisms isolated from cases of dysentery—apart from a determination of superficial resemblance in growth

characters on the usual nutrient media and their reactions in the presence of the more commonly employed sugars, —dextrose, lactose, and saccharose—have been largely identified by their supposed specific agglutination reactions in the presence of serum from dysentery patients and immune animals. That there are serious objections to placing too complete confidence in such measures alone, we believe will appear from our study.

The bacillus of acute dysentery, or diphtheritic colitis first described by Shiga (1) in 1898 as etiologically connected with the disease in Japan, and the bacillus isolated by Kruse (2) in 1900, from the same disease in Germany, have finally after much comparison and discussion of minute differences been recognized as identical species. Under the stimulus of Flexner's (3) work, the same species has undoubtedly been isolated from cases occurring in America, as is shown by the corresponding cultural characters and serum reactions of the organism isolated in New Haven, Conn., and known as culture "New Haven" (4). Flexner's culture isolated by him in 1899 while in the Philippines is not admitted by all observers to be identical with the preceding, although Kruse—aside from the general weaker growth of the Flexner bacillus, and minor differences in its agglutinating reactions, which are as a rule less marked in serum from dysentery cases than the reactions of "Shiga," "Kruse," and the "New Haven" cultures—could determine no specific differences, and concluded that the variation was not greater than that occurring among various true typhoid cultures. Shiga (5) in his latest article is practically of the same opinion, and claims to have gotten similar reactions to those given by Flexner's bacillus with cultures of his own, when these were grown for several generations in milk. From this he believes the Flexner

bacillus to be simply a slightly modified form of the true Bacillus dysenteriæ.

Martini and Lentz (6), however, and particularly Lentz (7) in a separate article on the cultural differentiation of these organisms, claim to have found not only discrepancies in agglutination, but cultural differences of fixed value in the Flexner bacillus, as brought out in mannit-litmus agar (Drigalski's medium). To a discussion of this point we shall return later.

The bacillus, to a consideration of which we invite your attention, was isolated from the contents of a section of the colon of a child dead of an acute diarrhœa. We are indebted to Dr. David Bovaird for the material, and as he is here this evening we leave it to him to tell you something of the clinical course of the disease, and of the pathological changes found at death.

Method of Isolating the Organism.—The method employed by us for isolating the organism was as follows: The contents and scrapings from the mucosa of the gut were diluted in broth and platings were made from this emulsion by transferring one to several loopfuls to the medium* recommended by one of us (Hiss) (8) for isolating the bacillus of typhoid fever. In this medium the colonies of the bacillus of Shiga do not form threads, as do the typhoid bacilli, and may usually be distinguished from the colonies of colon bacilli and allied forms, by being smaller and lighter in color. Transplants were made from the small, light colored, transparent colonies which were present, into tubes of the semi-solid medium described by Hiss (9) in 1897. By the use of this medium, which appears to us much superior to the

* Composed of agar, 15 gm.; gelatin, 15 gm.; Liebig's meat extract, 5 gm.; sodium chloride, 5 gm.; and dextrose, 10 gm. No acid or alkali is added. See reference No. 8, p. 156.

glucose agar recommended by other workers, organisms corresponding in character to the Shiga bacillus may be readily recognized. In this semi-solid medium when inoculated at 37° C., the bacillus of Shiga, possessing no active motility, does not spread out in the medium and cloud it; but its growth is confined to the original line of inoculation. Neither does the dysentery bacillus form gas in the medium, from the one per cent of glucose which is present. All colon bacilli form gas in the medium and are also distinguished by other characters, more or less marked, which depend upon their degree of motility. Typhoid bacilli are readily differentiated by clouding the medium uniformly,

If some such semi-solid medium as this had been employed, instead of dependence being placed on the microscopic test alone, much of the uncertainty and consequent discussion regarding the motility or non-motility of the bacilli described by Shiga and Flexner, it seems to us, might have been avoided.

As pointed out by Hiss some time ago, the character of the growth of the bacillus of Shiga in this semi-solid medium in tubes and its inability to form threads in the typhoid plating medium, were among the exceedingly few cultural characters until recently known by which this organism could be separated from the bacillus of typhoid fever.*

Description of the Bacillus.—An organism answering to the characters shown in the semi-solid medium by the Shiga bacillus was found to be present in the gut contents of our case, and was subjected to various tests to determine if possible its further identity with various cultures of Shiga's bacillus. Three cultures of this or-

* See reference No. 8, p. 165.

ganism sent to us by Prof. Flexner have been studied. These, as has been stated, are the cultures known as "Kruse," "Flexner," and "New Haven." "Kruse" is the culture isolated during an epidemic in Germany. "Flexner" is one of the cultures obtained by Prof. Flexner while in the Philippines, and "New Haven" sometimes referred to as "Seward," was from a case of dysentery occurring at New Haven, Conn.

Morphology.—The organism is a short, fairly stout bacillus, with rounded ends, in some cultures almost coccoid in form. It occurs singly, but often in pairs, and rarely short threads may be seen. There is a tendency in some preparations to occur in clumps. The bacillus stains with the anilin dyes, but as is the case with Shiga's bacillus, the staining of all the bacilli in a preparation is not equally intense. Near well stained individuals it is the rule to find many which have taken the dye very poorly. The staining of the protoplasm of individual bacilli is also at times irregular. The organism is decolorized by Gram's method. Spore formation has not been observed. The bacillus is not motile. Flagella stainings have not, so far, been made. No capsules have been noted.

In general morphology and staining reactions the organism answers most closely to that of the dysentery bacilli as represented by Kruse's culture. Flexner's bacillus is not so stout and is longer than ours, thus corresponding morphologically more closely to the bacillus of typhoid fever.

Cultural Characters.—The bacillus grows well on the the culture media, and corresponds on these in all essential respects to the Shiga bacillus. Gelatin is not fluidified; nor is indol, according to our tests, produced. On the usual nutrient broth, which is clouded uniformly

by the growth, a small pellicle may be formed after ten or twelve days at 37° C. When a fermentable sugar is present in the broth no pellicle is formed. The same observation holds good for typhoid and colon bacilli. This absence of pellicle is probably accounted for by the ease of obtaining oxygen from the sugar and subsequent inhibition of growth by acid production. A failure to recognize this fact has undoubtedly led to discrepancies in decriptions of organisms. Some facultative organisms, usually not forming pellicles will do so in sugar free media, or at all events grow more abundantly in the upper portions where the necessary oxygen is present. None of our cultures of true Bacillus dysenteriæ have, however, formed pellicles.

Fermentation Tests.—A study of the organism in dextrose, lactose and saccharose bouillon in fermentation tubes shows a complete correspondence with the bacillus of Shiga, as represented by the cultures "Kruse," "New Haven," and "Flexner." All of these organisms ferment dextrose with the formation of acid and a clouding of the open and closed branch of the fermentation tube. Neither lactose nor saccharose are fermented by any of those organisms, hence are not available for anaerobic growth and the closed branch of the tube remains clear. These bacilli are not able to grow anærobically in broth not containing some available carbohydrate or allied source of oxygen supply.

It has been the common practice in our laboratory for several years past to test organisms, especially those of the typhoid, Gärtner, and colon groups, on one per cent. mannit broth in fermentation tubes. A test of organisms in this medium has in our hands always served to differentiate cultures of Shiga's bacillus—"Kruse," "New Haven," and Flexner"—from typhoid bacilli. This

point was first noted about two years ago in our laboratory by Dr. Charles Norris, who in observing the fermentative activities of certain members of the typhoid and Gärtner groups, tested the cultures "Kruse" and "Flexner" sent to us by Dr. Flexner shortly after his return from the Philippines. Typhoid bacilli, Norris found, utilized mannit with acid production and clouded the closed branch of the fermentation tube, while the dysentery cultures could not ferment mannit, and did not grow in and cloud the closed branch of the tube, which in glucose free broth always remained clear. This fact was called to the attention of Prof. Flexner at that time. The organism isolated by us when grown thus in mannit does not correspond in its action to the cultures "Kruse," "New Haven" and "Flexner" but ferments mannit and clouds the closed branch of the tube as do the typhoid bacilli. In bouillon made from maltose of high purity it can, however, be differentiated from typhoid cultures. Typhoid cultures ferment maltose with acid production and cloud the closed branch of the fermentation tube, while our organism does not ferment maltose nor cloud the closed branch.

It is well to note that cultures of Petruchsky's Bacillus fecalis alkaligenes can be distinguished from dysentery, typhoid and allied bacilli, by its inability to ferment any known carbohydrate (at least, anærobically) or to cloud the closed branch of the fermentation tube.

Serum-Water Media.—A most striking differentiation of organisms belonging to the dysentery, typhoid, Gärtner and colon groups is afforded by a study of these organisms in serum-water media. This medium as a basis for studying fermentative activities was devised by one of us (Hiss) for use in the differentiation of pneumococci from streptococci.

The medium is composed of clear beef serum, one part, and distilled water, two parts.* This mixture can be boiled without causing a coagulation of the albuminous bodies present. The medium after boiling is an opalescent fluid. As was pointed out in the original communication on this subject, this medium is practically, if not absolutely, sugar free. The original monosaccharid content of the blood is rapidly destroyed after drawing, by the action of the so-called glycolytic ferment of the blood. That this is still active in serum may be demonstrated by adding small amounts of dextrose to sterile serum, and then allowing this to digest at 37° C., for an hour or so. Subsequent fermentation tests with organisms will show that the sugar has entirely or partially disappeared, according to the amount added, as determined by acid or gas production. In using the medium as a basis for fermentation tests it is necessary to boil it before adding the sugars, as many of these are acted upon by the enzymes of the blood,—diastase, maltase (glucase), etc.—This may also be easily demonstrated by digesting the sugar, especially dextrin and maltose, for a short while in the unheated serum water mixture, maltose being rapidly destroyed and split into dextrose, and dextrin quickly changed to maltose and then to dextrose. The medium is therefore made by first heating the serum-water at 100° C. for a short time, and then adding usually one per cent of sugar desired. To this is added litmus solution (Merck's highly pure litmus, 5% solution in water) in the proportion of one per cent. The medium is then tubed and may be sterilized at 100° C. for ten minutes on three consecutive days without fear of changes taking place in the sugar from the application of the heat.

* Beef serum, one part, distilled water, three parts, is better as it is less opalescent.

The sugars so far tested by us are dextrose, galactose, mannit, maltose, lactose, saccharose, and dextrin. These sugars serve eminently well to differentiate our organism from the dysentery bacillus of Shiga, and also to distinguish between these and organisms of closely allied characters, such as typhoid bacilli, B. fecalis alkaligenes, colon, and the organisms of the Gärtner group. This is shown by the following tests of organisms:

B. fecalis alkaligenes: None of the sugars are fermented; all of the media remain blue and unchanged.

B. Shigae: *Dextrose* and *galactose* are fermented with acid formation as shown by a reddening and solid coagulation of the medium. Mannit, maltose, lactose, saccharose and dextrin are not fermented, the media remaining blue and fluid. This is true of "Kruse," "New Haven" and "Flexner."

Bacillus Y (Hiss and Russel): *Dextrose*, *galactose* and *mannit*, are fermented with acid production, as shown by reddening and coagulation of the media. The fermentation of mannit differentiates this organism from B. Shigae, as represented by the cultures "Kruse," "New Haven" and "Flexner." The maltose, lactose, saccharose and dextrin tubes remain fluid and blue.

Bacillus "48": From stool of typhoid fever patient in 1897. Same reaction as above with the exception that the mannit medium is not coagulated throughout the entire depth, but shows a blue fluid layer at the top. This organism, however, is actively motile and thus further distinguished from "Y."

Bacillus Typhosus: *Dextrose*, *galactose*, *mannit*, and *maltose* and *dextrin* are fermented with acid production, as shown by a reddening and solidification of the media. *Dextrin* tubes first show a change to purple, and later coagulate in the lower portion leaving for some time a blue fluid layer above. Lactose and saccharose media remain unchanged and blue. The fermentation of maltose and of the dextrin medium serve to differentiate typhoid cultures from our organism and from the culture "48."

Bacillus "48" deserves further mention. It was isolated by Hiss in 1897 from the feces of a New York Hospital patient supposed to be suffering from typhoid fever, and was thought at first to be a true typhoid bacillus; but was subsequently looked upon as doubtful as it did not form thready colonies in the typhoid plating medium and, although clouding the tube medium uniformly without gas production, formed a thin pellicle at the surface of the medium. This is never the case with true typhoid cultures. It had been preserved in sealed tubes until a short time ago when it was re-examined and found to differ from typhoid bacilli in its action on maltose and dextrin. Although its sugar reactions correspond very closely to those of our bacillus "Y," it is as above noted to be readily distinguished from it by its motility.

Agglutinating Reactions.—Unfortunately we were not able to obtain a specimen of blood or serum from the patient from whose intestine our bacillus was isolated, so that a most important link in the chain of etiological evidence is lacking. A study of the agglutinating reactions of the bacillus have, however, been made with various normal and immune sera, and with the serum of

an animal immunized with the bacillus itself. The following interesting and, it seems to us, very important results have been obtained.

Normal Human Blood:—The results of tests against supposedly normal human sera were as follows:

Serum A. Adult man, in good health; no history of bad diarrhœic or dysenteric attack. This serum did not agglutinate the bacillus in any dilution.

Serum B. Placental blood. No history furnished. Positive reaction 1–20, two hours. 1–30, two hours was negative.

Serum C. Adult man in good health. Two years ago while in Porto Rico suffered from a prolonged attack of diarrhœa, which was not, however, considered dysenteric in character. Positive reaction, 1–80, in two hours. Negative, 1–100, in four hours. This serum on account of the previous diarrhœa probably should be excluded from the normal list.

Human Sera, Dysentery:

Serum A. From a soldier with chronic dysentery. Acute attack in the Philippines two years ago. Another attack subsequently in Porto Rico. Positive reaction with our bacillus, 1–20, one hour. "Flexner" did not react at 1–20. "Kruse," —1–10, —1–40; "New Haven," +1–10; +1–20; —1–40.*

Serum B. From a soldier with chronic dysentery. Sent to us from the military hospital in Washington. Acute attack of dysentery in the Philippines two years ago. Our organism reacted 1–40, one-half hour. "Flexner" gave no reaction. "Kruse" was positive 1–20; minus, 1–40, one and one-half hour. "New Haven" positive 1–20; negative, 1–40, one and one-half hour.

* Indicates a partial reaction.

Normal Horse Serum:

Bacillus "Y" +1–40, one-half hour: ±1–80.
"Kruse" +1–20; ±1–40.

Dysentery Horse Serum: From a horse immunized against culture "New Haven." Sent to us by Prof. Flexner.

Bacillus "Y" +1–400; ±1–500, twenty hours.
"New Haven" +1–100; ±1–200, twenty hours.
"Flexner" +1–40; —1–200, twenty hours.
Bacillus "48" —1–1; —1–50, twenty hours.
B. typhosus +1–20.

Normal Rabbit Serum:

Bacillus "Y" ±1–2; —1–10, 1–20, 1–100, two hours.

Typhoid Rabbit Serum: Highly immunized against typhoid. Exact titre against typhoid originally 1–20,000.

Bacillus "Y" +1–500; +1–4,000.
"New Haven" —1–20.
Bacillus "48" —1–25 and 1–100, twenty hours.

"Y" Rabbit Serum: This animal received several inoculations of boiled cultures of "Y". The serum agglutinated "Y" in dilutions as high as 1–500. "Kruse," "New Haven" and "Flexner" did not react in any dilution (—1–10; —1–500). Bacillus "48" also showed no agglutination. B. typhosus reacted at 1–80; ±1–100 in twenty hours.

Normal Beef Serum:

Bacillus "Y" +1–200; +1–320, four hours.
"Flexner" +1–10, —1–20.
"New Haven" +1–10; —1–20.
"Kruse" +1–10; —1–20.
B. typhosus +1–40; +1–80, twenty hours.

The results of these serum tests are important and indicate to us, after a careful search of the literature of dysentery, that we are in all probability dealing with an organism not hitherto described. It is readily seen from our tests that "Y" agglutinates with dysentery serum from human infections and from immunized animals in most instances quite as well as, and in some better than do bacilli fully identified in all respects with the bacillus of Shiga. The various dysentery-like baccilli described by other observers, although showing some agglutination in the presence of dysentery sera, have been agglutinated to a markedly less degree than true dysentery bacilli, and for this reason alone have in several instances been excluded from recognition as fully identical with Shiga's bacillus. This is true of the organism described by Kruse as the bacillus of pseudodysentery of the insane. The organisms placed in this class were, according to him, so far as he could determine, in all respects similar to his and Shiga's dysentery bacillus in cultural characters. They were differentiated by not agglutinating with true dysentery serum, but with the serum from insane patients. The true bacillus of Shiga on the other hand did not agglutinate with these sera in the same dilutions. These pseudodysentery cultures were agglutinated in normal sera in 1–50, which is higher than that usually shown by the true dysentery bacillus. Kruse has found that his true dysentery organism agglutinates in normal sera as high as 1–20, and exceptionally 1–50. In his later paper, however, he thinks this may have been due to previous infection.

Another point of interest is the high agglutination of our bacillus with sera from animals immunized against typhoid. Here "Y" shows a very close relationship to the bacillus of typhoid. This bond is strengthened by the

fact that typhoid cultures also show fair agglutination in "Y" immune serum. Both Shiga's bacillus and typhoid call forth agglutinins which have an affinity for the receptors of our bacillus "Y". "Y" immune serum on the other hand contains some agglutinins active for typhoid bacilli but none, so far as our experiments go, which affect Shiga's bacillus.

"Y" and typhoid bacilli, therefore, call forth some agglutinins in common or have receptors capable of anchoring certain agglutinins of each. "Y" it may be, anchors all of the agglutinins called forth by Shiga's bacillus and probably other agglutinins normally present in the serum tested. This is indicated by its higher agglutination in the dysentery horse serum than that shown by the dysentery cultures themselves. This is the case; or it has fewer receptors that have to be satisfied before agglutination takes place. The same phenomenon is probably illustrated by our organism and typhoid bacilli in normal beef serum. There are agglutinins present in the serum active for both typhoid and "Y". Our organism, is however, agglutinated in much higher dilutions than the typhoid bacillus, showing that there is much more "Y" agglutinating substance present, or that "Y" is agglutinated with fewer receptors satisfied than is the typhoid bacillus.

The action of beef serum is important as it may be found to afford a practical means of differentiating true Shiga bacilli from organisms of the "Y" type. Shiga's bacillus as above noted does not agglutinate in beef serum, to an appreciable degree.

These facts brought out in regard to the serum reactions of "Y", dysentery, and typhoid bacilli have important practical bearings. Unless careful studies are made of all organisms resembling Shiga's bacillus, gross

errors may occur in serum diagnoses based upon the use of such organisms in testing reactions; and errors may also occur in identifying such organisms themselves by simple tests against dysentery serum. This is illustrated by the fact that "Y" agglutinates better even than the true Shiga bacillus in dysentery serum, and unless true Shiga bacilli are tested against immune "Y" serum they may not be differentiated from each other. It is more than probable that organisms corresponding to "Y" have already been isolated and not recognized as distinct from Shiga's bacillus. This may then lead to the converse mistake of establishing the identity of a dysenteric or diarrhoeic attack with true dysentery by agglutinations obtained by the use of a bacillus differing from the supposed etiological organisms.

These remarks are not meant to convey the impression that "Y" itself is not or may not be found to be etiologically related to some form of dysenteric or diarrhoeic disease, but are intended to bring out the fallacy or relying solely upon serum reactions in identifying either disease or organism. It is fortunate, therefore, that the study of cultural characters has aided us in the recognition of differences in these organisms and may be of further service in the recognition and grouping of new organisms as they are discovered.

It seems to us in the face of our knowledge of the discrepancies in degree of agglutinability of such well-known organisms as typhoid bacilli, which not only show variations among the different cultures, but in the same species under practically non-determinable culture conditions, that we should hesitate before placing too much confidence in differences determined by serum reactions alone, but should endeavor in all cases by careful and ex-

tended study to establish their identity or dissimilarity by every cultural and biochemical test available.

Arguing from the analogy of our work on immune animal sera, an infection due to a bacillus of the "Y" species might, if tested against typhoid bacilli, give a reaction in a dilution considered diagnostic, and sera from both dysenteric and typhoid patients when tested against "Y", would certainly give reactions in high dilutions. Conversely, "Y" might be identified either with typhoid or Shiga bacilli if dependence were placed on serum reactions alone in its recognition.

Pathogenicity.—Our bacillus is pathogenic for both rabbits and guinea-pigs, but not markedly so. No characteristic lesions have so far been noted. Some hyperaemia of the small intestine is apparent. Rabbits may be readily accustomed to large doses of living cultures without showing a marked loss in weight.

As noted earlier in this paper, Lentz in a cultural study of a large number of true Shiga bacilli and other more or less closely allied organisms isolated from dysenteric patients, claims to have been able to separate by the use of a mannit litmus agar the cultures, which he designates as "Flexner I," "Flexner Manila" and "Strong," from the true bacillus of Shiga and Kruse. The results of his tests indicated that grown in this medium, Shiga's bacillus does not give rise to changes in the medium, with the exception of a decolorization of the medium in its lower layers, the medium remaining of the normal blue or deep purple in the upper section. In the media inoculated with the "Flexner" and "Strong" bacilli, the color changes to a distinct red due to acid production.

We have endeavored to repeat Lentz's experiments. The medium used by him in his earlier experiments was

made according to the formula given by Drigalski for his typhoid isolation medium, with the substitution of mannit for the lactose. In Lentz's article on *Dysentery* in *Kolle* and *Wassermann's Handbuch der pathogenen Mikro-organismen* (p. 321), the method of preparation is somewhat simplified. This last method is the one followed by us.

At the present moment we are unable to say whether our culture "Flexner" is one of the organisms designated by Lentz as "Flexner I" or "Flexner Manila".

The tests of our culture "Flexner" on Lentz's medium do not agree with his published results.* Flexner's

* Since writing the above, I have had the opportunity, through the kindness of Dr. Flexner, of examining the cultures "Flexner Manila (Harris)" and "Flexner Manila (Gray)" and Strong's Manila culture. These are the cultures examined by Martini and Lentz, and the culture "Flexner" examined by us, according to our tests, is not one of them. Both of Flexner's Manilla cultures, as well as that of Strong, differ from the true Shiga bacillus in fermenting mannit, as shown by acid production. Strong's bacillus seem to be identical with our organism "Y". Flexner's Manila culture, as well as one of his Baltimore cultures (sent to me by Dr. W. H. Park) is further differentiated from Shiga's bacillus and from our bacillus "Y" and Strong's culture by fermenting maltose. Saccharose is also fermented with acid production by Flexner's Manila cultures and also the culture "Baltimore." My tests also indicate that these bacilli will produce acid in the presence of dextrin. Their fermentation of saccharose and the absence of active motility separates them from B. typhosus. Their fermentation of maltose, saccharose and dextrin is slower than their action on dextrose and mannit, and may not be apparent for some days. Control saccharose tubes inoculated with B. typhosus show no change, so that this cannot be attributed to the presence of dextrose derived from the splitting of saccharose in the preparation of the medium. Control tubes of maltose and dextrose inoculated with Bacillus "Y" and Shiga's bacillus also remain blue and unchanged.

We have then, apparently, three groups of organisms. One represented by the true Shiga bacillus, fermenting dextrose and some other monosaccharids: one by our organism "Y", fermenting dextrose (monosaccharids) and the alcohol mannit: and finally a group represented by the more active fermenters, Flexner's Manila cultures and the culture "Baltimore," organisms which split dextrose, mannit, maltose, dextrin, and saccharose with the production of acid, as indicated by the reddening and coagulation of the serum-water sugar media.

This study shows plainly the inadvisability of relying upon superficial

bacillus gives identically the same reaction in his litmus mannit agar as the results obtained when "Kruse" and "New Haven are tested. The culture medium of Lentz, which is used in tubes and inoculated by central puncture, in all three instances became slightly reddened below but remained blue at the surface. The medium in Lentz's experiments with Flexner's bacillus reddened.

Our bacillus "Y" and the organism "48" as well as true typhoid bacilli, when grown in the medium give rise to a reddening of the medium throughout its entire depth. These results were what we naturally expected from our knowledge of the metabolism of these organisms. "Kruse," "New Haven" and "Flexner" do not utilize mannit either in the presence or absence of oxygen or give rise to acid from it. Bacillus "Y" and "48" and typhoid bacilli ferment mannit with rapid production of acid, and hence redden the entire medium. This was to be expected from their fermentation tube reactions and the results of our experiments with serum-water plus mannit.

An explanation of these discrepant results may possibly, however, be found in the fact, which seems to have escaped the notice of both Drigalski and Lentz, that the medium to which they add sugars, such as mannit, lactose, etc., to be tested, is *not* glucose free. No mention is made of the removal of muscle sugar from the nutrient infusion used by them, nor is this recommended in the directions for preparing the medium. The presence of a fermentable substance, not mannit, in Lentz's medium, is clearly demonstrated by the reddening and possible subsequent clearing of the lower portion of the medium

characters or agglutination tests alone in the identification of an organism, or on the serum tests in the determination of the special character of a disease, and also lends support to our supposition that organisms differing from the true Shiga bacillus have been isolated and confounded with this organism.—*Hiss.*

when tested with our cultures "Kruse," "New Haven" and "Flexner" which do not ferment mannit. That the medium remains blue at the top may simply indicate that in the presence of oxygen the alkali production neutralized or exceeded the production of acid.

We have made up samples of the medium from sugar free meat infusion and find that in tests of cultures "Kruse," "New Haven" and "Flexner" no reddening of any portion of the medium occurs. Furthermore, when the medium is used soon after heating and oxygen driven off, the growth is confined to the surface, or upper parts of the inoculation line, since these organisms do not grow anaerobically in the presence of mannit in otherwise sugar free media. In tests of mannit fermenting organisms such as "Y," "48" and typhoid bacilli, the glucose free mannit medium is found reddened throughout, and growth has taken place along the entire length of the inoculation puncture.

None of the results of Lentz's sugar tests, although they may be roughly indicative, can be scientifically reliable so long as the medium used by him contains this original sugar, and the justice of our criticism of his method is evident in the discrepancies noted by Lentz* himself between the action of colon bacilli on media made by him and the results obtained by Drigalski with colon bacilli. Furthermore, slight differences in the initial reaction of the medium (alkali content) which are unavoidable with their method of neutralization and alkalinization, might also determine differences in their color reactions.

Resume.

To review briefly: We have in this paper described

* See foot note by Lentz. *Zts. f. Hyg.* 1902, Bd. XLI, h. 3, pp. 560, 561.

an organism from a case of fatal diarrhœa. The organism agglutinates in high dilutions with the serum from dysentery patients and animals immunized with Shiga bacilli. Formerly this bacilli "Y" could not have been differentiated by valid culture tests from the bacillus of Shiga; and, the mannit test being unknown, might easily have been confounded with Shiga's organism unless its agglutinating reactions had been carefully studied in such a serum as that from typhoid immune animals, or from the normal beef, in neither of which the true Shiga bacillus agglutinates in appreciable dilutions.

The bacillus described by us is also closely related to B. typhosus, both in agglutinating reactions, and in the usually observed cultural characters, but can be separated from it by the absence of motility and by its reactions in the maltose and dextrin media employed by us.

This bacillus apparently differs from all hitherto described dysentery-like or "pseudodysentery" bacilli, in its agglutination in high dilutions of dysentery serum, an agglutination in some instances more marked than that shown by the true Shiga bacillus. A description of this organism—apart from its connection, which may or may not be etiological, with the case of diarrhoea—has seemed to us important, since tests of its ability to agglutinate —too often alone relied upon—might lead to its being confounded with Shiga's bacillus. A subsequent use of such an organism for serum diagnosis might thus give rise not only to confusion in determining the "specific" character of a disease, but to untoward results in the application of serum therapy.

Its etiological significance we have not been able to determine but its close physiological connection with the dysentery bacillus on one side, and the bacillus of typhoid

fever on the other, make it not improbable that further research will show it to be associated etiologically with certain diarrhœic or dysenteric attacks.

Discussion.

Dr. David Bovaird gave details of the case from which the organism described by Dr. Hiss had been isolated. The patient was a child of about one year, who suffered from an attack of enterocolitis. The temperature was not high, never over one hundred. There was persistent diarrhoea, from four to six stools every day. For the most part they had been green and contained mucus. There had been no mention of any blood in the stools. The child lost strength progressively and died in two weeks. Dr. Bovaird considered that Dr. Hiss' results were of the utmost importance. He said that it was only a short time since the Society had listened to Dr. Flexner describe the finding of the bacillus of Shiga in approximately fifty cases of dysentery in children, and since that occasion there had been numerous reports of the finding of the bacillus in diarrhoeas of children in various parts of the country. Efforts had already been made to develop a serum which would be of use in the treatment of these cases. He had but recently read the report of the work of Gay, of the University of Pennsylvania, who thought he had been able to produce such a serum. Since, therefore, it was evident that we were approaching the treatment of some of these cases by sera, it became very important that the diagnosis in all cases should be very exact before such treatment was undertaken. Such observations as Dr. Hiss' were therefore of great value, in indicating a possible source of error. Further investigation would be required to de

termine the full application of Dr. Hiss' observations. If the Shiga bacillus were found to be the specific cause of many of the diarrhoeas common in children, the association with the term dysentery would be a source of some difficulty and confusion. That term had not been seen in pediatric writings for some years, and there was no clear conception of the clinical picture which it should call up. Certainly there were marked differences betwen the diarrhoeas common in children during the summer and the condition in adults designated as dysentery. If it were consistent with the facts, it would be an advantage in discussion to employ the term colitis or enterocolitis, which would be much more definite and clear.

Dr. W. H. Park said that only the night before Dr. Smith had inoculated all these different bacilli in nutrient agar to which litmus and mannit had been added. There was no question that on this medium which had a trace of meat sugar, the Shiga bacillus and the Flexner New Haven culture stood out perfectly clearly from the rest in not having produced appreciable acid. Although the elimination of the meat sugar might be insisted on so as to judge what effect the bacilli had on mannit, yet the marked differences between these bacilli and all the others was just as important in showing differences between the bacilli as if the medium had contained only mannit. Dr. Park did not think it correct to speak of developing in this country a new serum for dysentery. Shiga had used the serum in a number of cases and attempts made here so far had been simply copies of what he had already done. Shiga himself had only employed the usual methods. He thought it was a mistake to go too fast in applying the results obtained by those working under Dr. Flexner at Baltimore, to ordinary summer diarrhoeas until it was proven whether

all these bacilli were really essentially identical and also whether it might not be true, as seemed probable, that the conditions at the Wilson Sanatorium were unusual in the amount of infection which existed with dysentery-like bacilli.

Dr. Hiss said that he wished to say that the tubes of Lentz's medium shown contained media made according to Lentz's formula, and also mannit media made from sugar free infusion. If held against the light one could see that the sugar free tubes showed no growth except at the surface, and not along the line of inoculation.

Dr. E. Libman said that he thought there were several points in Dr. Hiss' paper deserving of corroboration. He had noticed as had Dr. Hiss that the medium described by Drigalski and Conradi was not made sugar free and he had therefore hesitated about using it. As regarded the value of agglutination reactions for the diagnosis of dysentery, he had examined a number of bloods from cases not having a history of dysentery to ascertain whether the reaction was specific; for he thought the reaction, if specific, could be used to establish the identity of certain conditions which appeared to be sequelae of dysentery (the patients at the time of observation not having evidences of dysentery). He had examined the blood from ten cases taken at random in the wards. The reaction was absent with the Flexner bacillus in all but two cases. One case of auto-intoxication gave a good reaction at 1–50; and a case of rheumatism gave a good reaction at 1–100 in twenty minutes. Possibly in higher dilutions the agglutination tests might have been specific. He was interested in Dr. Hiss' remarks about the occasional absence of clump reactions in low dilutions when present in high dilutions. He had himself observed this with immune typhoid serum. This

was an important point practically. He had examined the blood of two cases of typhoid fever which had given only a slight reaction at 1–20, but a complete reaction at 1–50 and 1–100. As only complete reactions could be considered in making the diagnosis of typhoid fever, he believed that when a blood does not react in low dilutions, higher dilutions should be tried. It seemed probable from the paper just read that just as in cases of paracolon infection the organisms showed variations, the same was true of dysentery, and in the same was the clinical picture of typhoid fever was due to a variety of organisms. Dr. Libman said he had recently seen a case of typhoid fever with perforation in which the Widal reaction was absent. Typhoid bacilli could not be found, but in the mesenteric lymphnodes, at the autopsy, the bacillus fecalis alkaligenes was found. Serum reactions could not be tried. He did not wish to draw any conclusions from this finding, but believed it was of interest because this organism had been discovered by Petruschky alone or with the typhoid bacillus in cases having the clinical picture of typhoid fever.

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A METHOD OF SEPARATION OF COLONIES OF SHIGA'S BACILLUS FROM THE COLON BACILLUS.

E. K. DUNHAM, M.D.

Dr. Dunham gave an outline of studies of the influence of the physical properties of gelatin upon the appearance of colonies grown on plates. The physical characters chiefly noted were: the viscosity, the melting point, and the penetrability or spissitude. It seemed to him that a knowledge of the viscosity and the melting point was sufficient for a practical guide to the influence of the gelatin on colonies. To obtain a differentiation of colonies it was found desirable to grow them in a soft jelly, either by making a soft jelly to start with or by using a stiff jelly and incubating the plates at a high temperature. The size of the colonies was smaller if the plate was allowed to stand and harden at the room temperature before being put into the incubator, considerably larger if put at once into the incubator. In twenty-four hours the colonies prepared in the latter way were larger than colonies forty-eight hours old in a similar but softer jelly, grown at the same temperature, but chilled before incubation. In a soft jelly the typhoid bacillus formed threading colonies. The colon bacillus in a very soft

jelly does not produce single colonies, but a collection of small colonies. The dysentery bacillus always produced a perfectly spherical colony except in jelly that was so soft as to be semi-fluid. For practical purposes the dysentery bacillus produced a perfectly spherical colony, easily distinguished from the colon and typhoid, more difficult to distinguish from the paratyphoid. It seemed therefore that in plating feces it would be very easy to ignore the colon bacillus and pay attention only to the spherical colonies. This would reduce the difficulty of finding the dysentery bacillus and could easily be done in twenty-four hours.

ON THE INTERPRETATIONS OF REACTIONS OF AGGLUTINATION AMONG THE BACILLI OF DYSENTERY.

WILLIAM H. PARK, M.D.

Dr. William H. Park gave the results of some experiments in agglutination reactions done by Dr. Collins and himself, in the course of which they had struck some things which he considered very remarkable. These were along the lines of Dr. Hiss' and Dr. Libman's remarks, except that they were perhaps more striking. He first spoke of normal goat serum and the necessity of knowing the serum agglutination results before the animal was inoculated. The serum from different goats varied widely. Experiments were made with the Shiga, Flexner Manila and New Haven bacilli, and with the bacillus isolated by himself and Dr. Dunham at Seal Harbor, Mt. Desert, and also with a bacillus furnished by Dr. Wollstein from one of a series of cases of dysentery oc-

curring in the Foundling Asylum in New York City. In normal serum from goat (3) the Shiga, Flexner Manila and New Haven bacilli agglutinated perfectly up to 1–200 within from one to one and one-half hours, while the Seal Harbor bacillus refused absolutely to clump in any dilution above 1–10. After the goat had been inoculated with three injections of the Seal Harbor bacillus the tests showed that all the bacilli mentioned were clumped in dilutions somewhat above 1–200 except the Seal Harbor. This agglutinated in dilutions only up to 1–50. This at first seemed unexplainable until reference to the results of the normal serum tests already spoken of showed that the slight reaction of the Seal Harbor bacillus in the serum of goat (3), after moderate injections with it, was due to the fact that the goat originally had almost no agglutinating substance for this bacillus, while having much more for the others. Such experiences proved the necessity of knowing the properties of the animal's serum at the beginning, before injections of bacteria or blood were made. After five injections of Flexner's Baltimore cultures the horse serum clumped all the bacilli except the Shiga and Flexner New Haven cultures which did not clump at all in dilutions higher than 1–75. All the others clumped up to 1–1000. A bacillus obtained by Dr. Park from three persons suffering from chronic dysentery, which in sugar fermentation was closely allied to the colon bacillus, clumped in this Baltimore culture horse serum in dilutions up to 1–10,000.

Curiously enough this bacillus had clumped in every dysentery serum in which the Flexner cultures other than the New Haven were clumped. In two human typhoid immune sera the Shiga and New Haven cultures did not clump at all, while the New York, Manila and

Baltimore cultures clumped in 1–50 and pretty well in 1–100. In this serum the Seal Harbor and Tuckahoe bacilli fell in the Shiga group, as did the bacillus from the chronic dysentery case. Tests with serum from a patient with a staphylococcus abscess gave no reaction with the Shiga, New Haven or Seal Harbor cultures; but an absolute reaction in dilutions of 1–100 with the chronic dysentery, Baltimore, Wollstein, and colon bacilli. Throughout all the agglutination tests the Shiga and New Haven bacilli showed divergence from the others. They had not been agglutinated by the agglutinating substances produced by or acting upon the others. The results of the tests with the colon-like bacillus which agglutinated in dilutions of 1–10,000 in the serum of a horse immunized with Manilla and Baltimore dysentery bacilli seemed to show that simple agglutinations even in dilutions much higher than those effective upon the organism used to immunize the animals were not enough to prove relationship between bacteria. In alkaline peptone mannit litmus nutrient agar the New Haven, Tuckahoe and Shiga cultures did not change the blue color in twenty-four hours. In all the other cultures production of acid occurred. The Flexner Manila, Baltimore, Seal Harbor and New York cultures produced indol in twenty-four hours in peptone water, while the Shiga and New Haven cultures did not, even in fourteen day's growth. Both in indol and acid productions therefore, as in agglutination reactions, the Japan and New Haven cultures were separated from the Manila, Baltimore, New York and Seal Harbor cultures.

Discussion.

Dr. Charles Norris asked by what method Dr. Park had made these agglutination reactions and what

limits he used in determining them. The results seemed to him very extraordinary. Had Dr. Park used the microscopic method? He had himself got hold of a highly immune serum but had never been able to obtain higher agglutination reactions than 1–100 or 1–150. By the microscopic method he had got reactions up to 1–300. He had been particularly surprised by Dr. Flexner's remarks that he had got agglutinations in dysentery cases up to 1–1,000. He had never been able to obtain any nearly so high. The thing might depend upon what one called an agglutination reaction.

Dr. Park said that with the goat serum the complete reactions were both in the test tube and hanging drop. The dilutions of the immunized horse serum were mostly tested by the hanging drop methods. Up to three or for hours the results obtained with the two methods were identical. For longer times the tube method gave the more accurate results. All the results were obtained within two or three hours, when the hanging drop was used, most of them within two hours. The complete precipitation of the bacilli in the tubes when agglutination took place was slow, requiring a number of hours. The cultures used were broth cultures, three to six hours old. Equal parts of the diluted serum and of the broth cultures were mixed together.

Dr. E. Libman asked whether Dr. Park had found any thread reactions. In his own experience, under certain conditions, the same organism after it had been grown for a length of time in various media would often change as regarded its reaction. He had observed a paracolon bacillus which at first gave only a thread reaction with the patient's blood and not with any other blood, but which after a time gave a clump reaction with

almost any blood tried against it. He asked whether Dr. Park had tried the reactions against any large number of normal sera.

Dr. Park said that in testing blood sera from patients in dilutions of 1–10 to 1–50, he had found a great many thread reactions after twelve to eighteen hours. In high dilutions with the sera of immunized animals he had seldom found any.

Dr. P. H. Hiss said that in connection with Dr Park's remarks he was reminded of Shiga's latest article in which Shiga described the growth of his own bacilli in milk, by which means he got precisely the same reaction with the true Shiga bacillus as with the Flexner bacillus. Dr. Hiss said that the results of his serum tests did not seem to show a correspondence between the Flexner bacillus which he had tested and the organisms "Y" which he had described. The "Y" had the power of agglutinating in very high dilutions, whereas the Flexner tested by him always agglutinated much less readily, in dysentery sera, and in other sera against which it was tested. He also said that the results of serum tests both in America and Europe indicated to him that one at least of Flexner's organisms did not react as did the Flexner organism tested by Dr. Park,—that usually it showed a markedly less agglutination than "Kruse," "Shiga" and "New Haven" in dysentery sera, from animals not immunized against itself.

Dr. Park said that he thought Dr. Hiss had misunderstood Shiga's article. He had had the same idea after first reading it, but after a careful re-reading, it became plain that Shiga said that the pro-agglutinoid zone was changed by the growth in milk, but not the agglutinating reactions. The Shiga bacillus still failed to remove the substances agglutinating the Flexner Manila culture and vice versa.

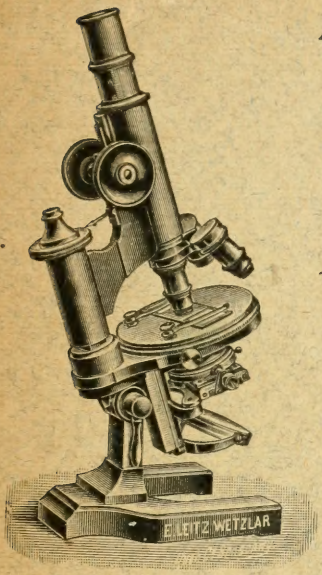
ON THE NATURE AND ORIGIN OF BLOOD PLATES.

L. B. GOLDHORN, M.D.

Dr. Goldhorn presented photographs and read a short paper on some observations which he had made on the formation of blood plates. After reviewing the literature, especially the work of Deetjen, he said that the reason that there were so many different opinions on the subject of blood plates was that in ordinary methods of heat fixation they were not well stained. Only in preparations in which very little heat was used or in which the fixation was chemical were the blood plates present in large numbers. They were more noticeable when strong dyes were used and were especially well demonstrated by the chromatin mixtures with eosin and polychrome methylene blue. One of the essentials was a thin smear. With the chromatin stain a central body could often be distinguished, of a bright red color sometimes compact, sometimes granular, surrounded by a central zone, always capable of being stained by basic dyes. He said there were several forms of blood plates, one devoid of chromatin granules; one with scattered granules; and one showing a compact mass of granules. In certain diseases abnormally large platelets were seen, especially in malaria. The platelets were regularly found in the blood in intimate relationship with the fibrin. Where there was no fibrin, there were no platelets. His conclusions were that the platelets were distinct and independent elements of the blood. They were not of a degenerative nature. There were several distinct types, all circular except for the fusiform variety. The size varied in health and disease. As to the origin of the plates the speaker said he had never had any evidence that they were derived from erythrocytes.

Discussion.

Dr. EWING said that he had followed the discussion of the origin of blood plates for a number of years and had come to conclusions contrary in certain respects to those of Dr. Goldhorn. He had found that there was very convincing evidence to show that the blood plates originated from red blood cells and were sometimes stainable within them. He thought that there was abundant reason to believe that the platelets resulted from the extrusion of a nuclear substance from the red cells. By far the weight of evidence was in accord with this view. He thought that Dr. Goldhorn had brought out some very important facts. It seemed possible that there was more than one variety of blood plates in human blood, and Dr. Goldhorn's minute descriptions of their morphology were an important contribution favoring this view. He himself had been entirely unable to distinguish in human blood more than one variety.



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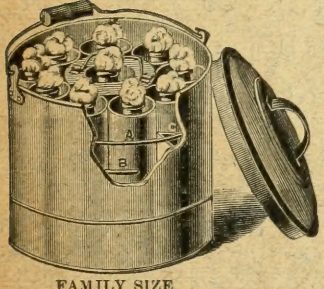

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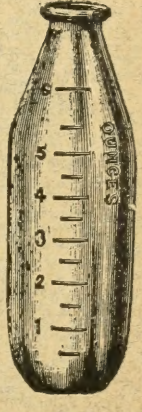

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